X-ray emission spectroscopy as a probe of electronic structure in d^{10} metal ions

A possible role for soft x-ray XES in studies of Zn proteins

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Zinc in Biology

- Zinc is the most common "trace" element
- Zinc is the only metal that is known to be required by at least one enzyme in every major class of reactivity
- Zinc levels are tightly regulated
  - Total Zinc (from *E. coli* to human cells) is ca. 10^{-4} M
  - Free Zinc is estimated at < 1 ion / cell

Why should biology want to use Zn?

- Good bio-availability
- Redox inert (safe in presence of O_2)
- Thermodynamically stable, e.g., $Zn(SR)_4^{2-}$
- Rapid ligand exchange
- Good Lewis acid
- d^{10} configuration
  - No geometric preference
  - Coordination sphere can easily be expanded

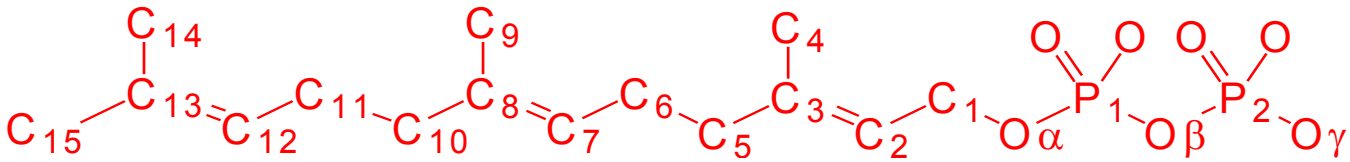
Structural questions about Zn proteins

- Ligation
- Bond lengths
- Geometry

Structural probes for Zn proteins

- Crystallography
- ^{67}Zn NMR
- X-ray spectroscopy
  - EXAFS
  - XANES

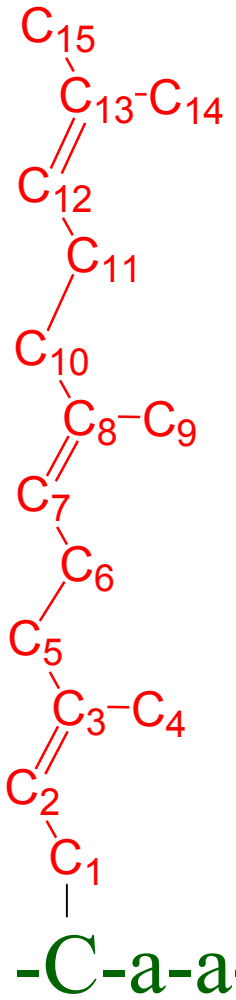
Protein Farnesyl transferase



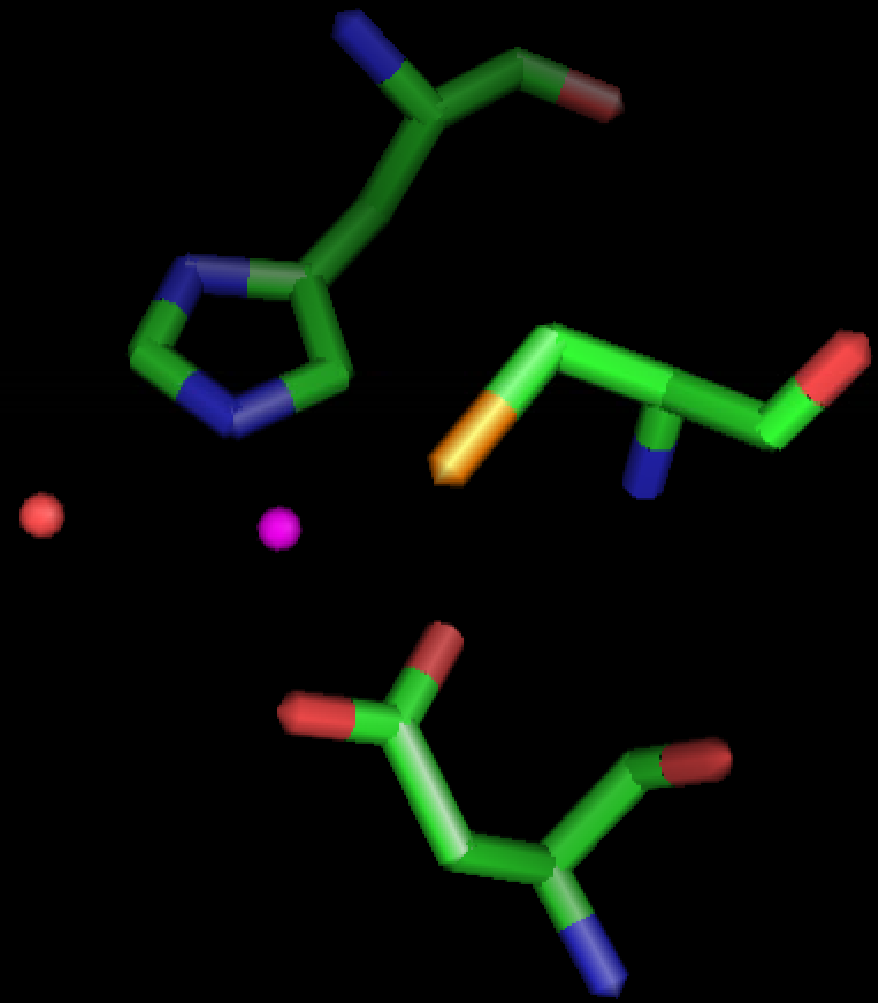
FPP

CaaX, X=S,M,Q,C,A

Peptide

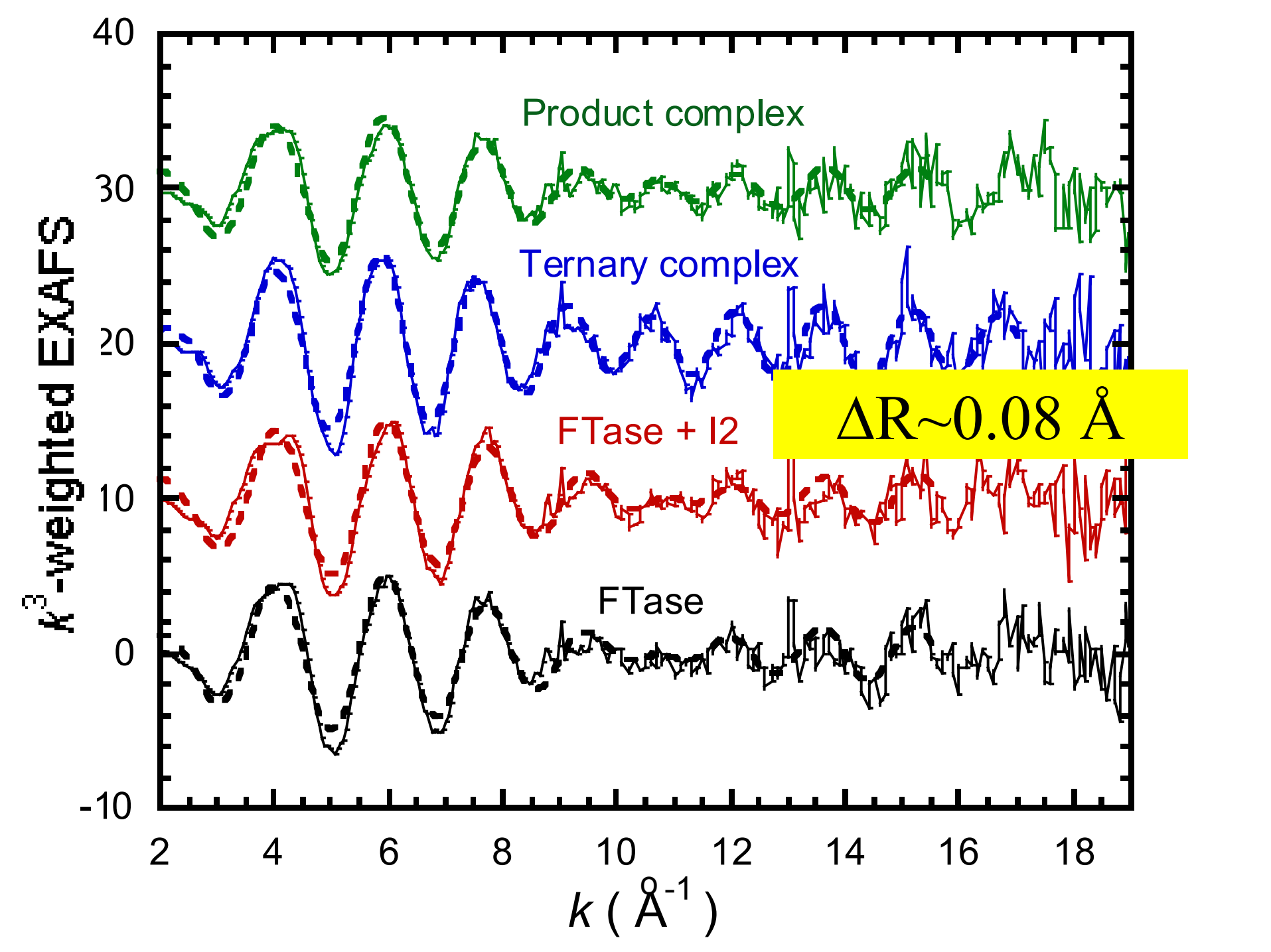


Protein Farnesyl transferase

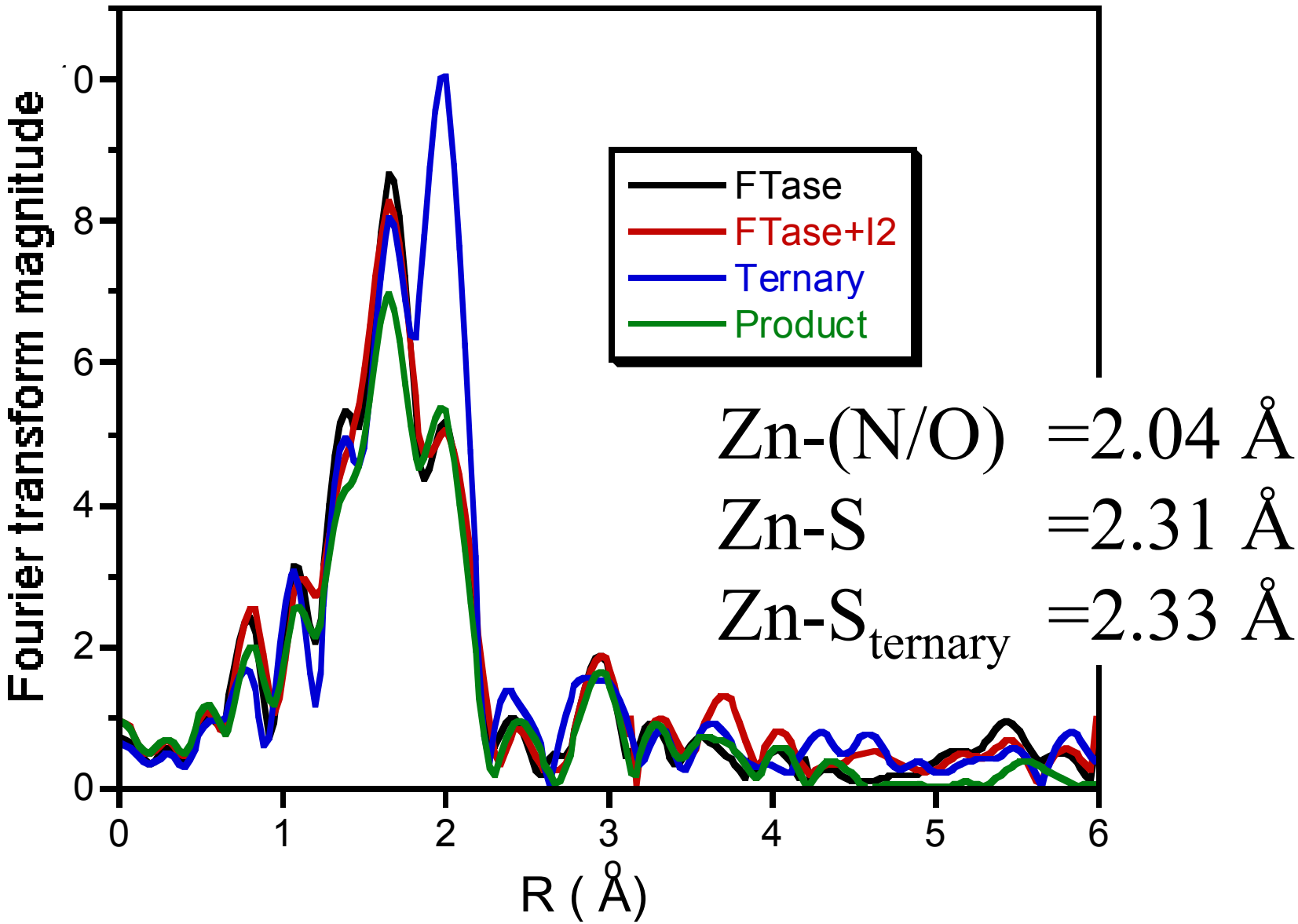


Ten FTase crystal structures are known

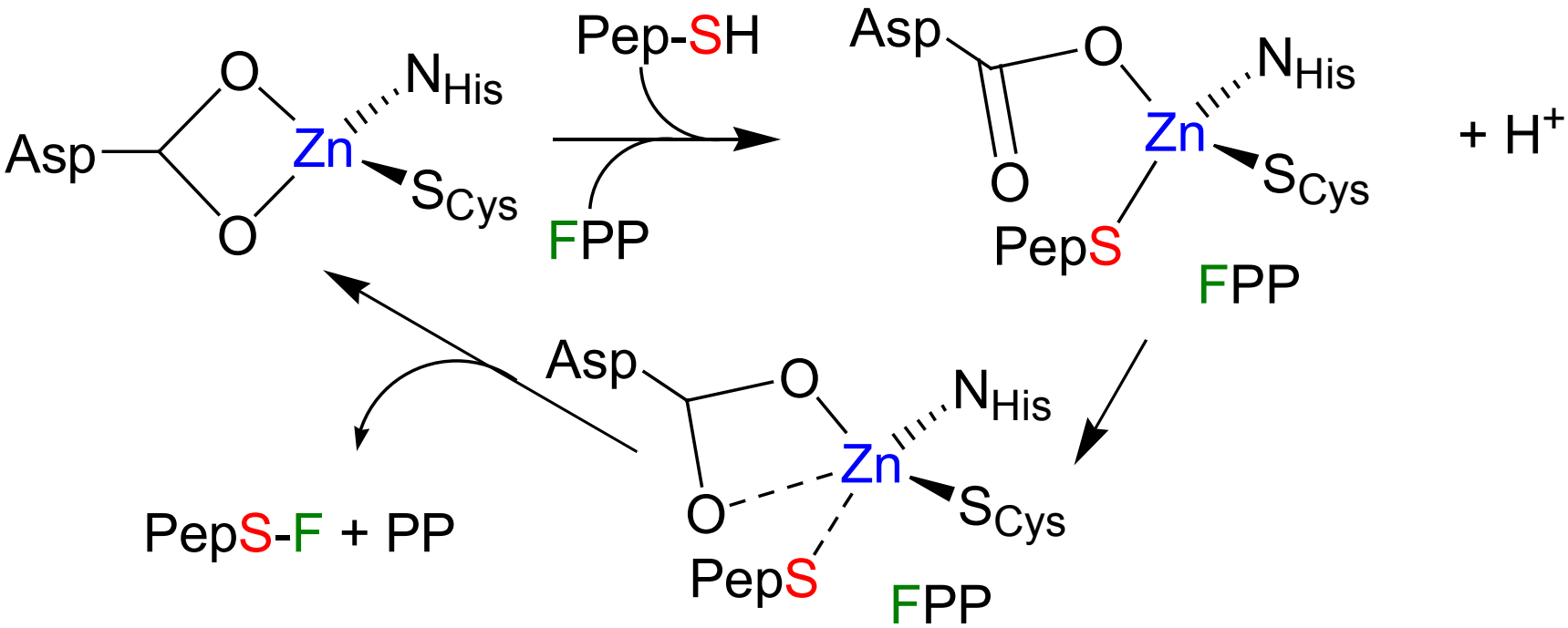
	Zn-S Pep	Zn-S C_{299}	Zn-O H_2O	Zn-O D_{297}	Zn-O D_{297}	Zn-N H_{362}
FTase	--	2.22	2.74	2.00	2.56	2.48
+FPP	--	2.27	3.22	1.99	2.03	2.10
	--	2.42	NA	2.38	3.05	2.64
Ternary	2.48	2.21	--	1.90	2.45	2.24
	2.40	2.26	--	1.99	2.61	2.18
	2.35	2.21	--	2.08	2.55	2.17
	2.41	2.33	--	1.97	2.53	2.21
	2.75	2.29	--	2.22	2.67	2.34
Product	2.66	2.27	--	2.06	2.42	2.18
Product+ FPP	--	2.30	--	2.13	2.42	2.25


Product complex
Ternary complex
FTase + I2
FTase
ΔR~0.08 Å
k^3-weighted EXAFS
k (Å^{-1})
40
30
20
10
0
-10
2
4
6
8
10
12
14
16
18

Zn is 4-coordinate; peptide sulfur binds only in ternary complex

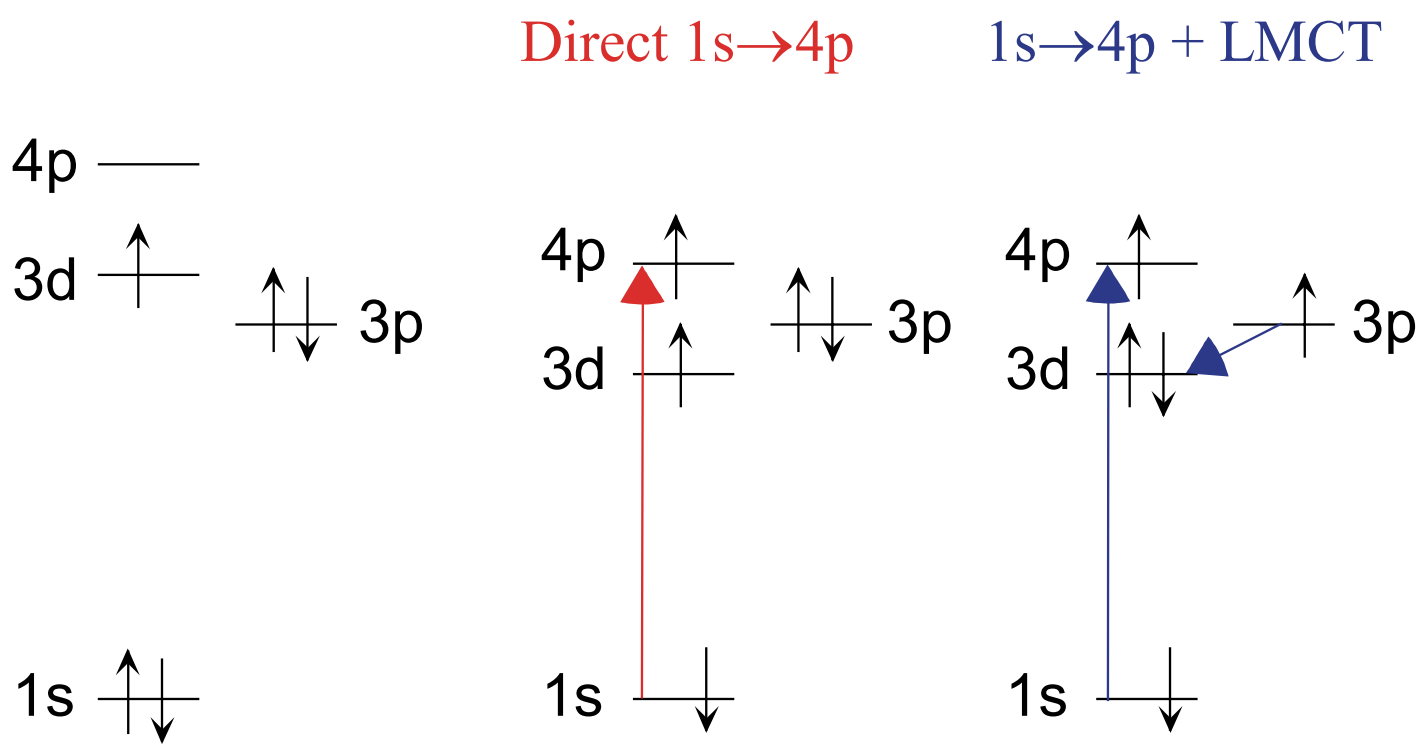


Carboxylate shift may play an important role in activating peptide thiolate



Electronic structure from XANES

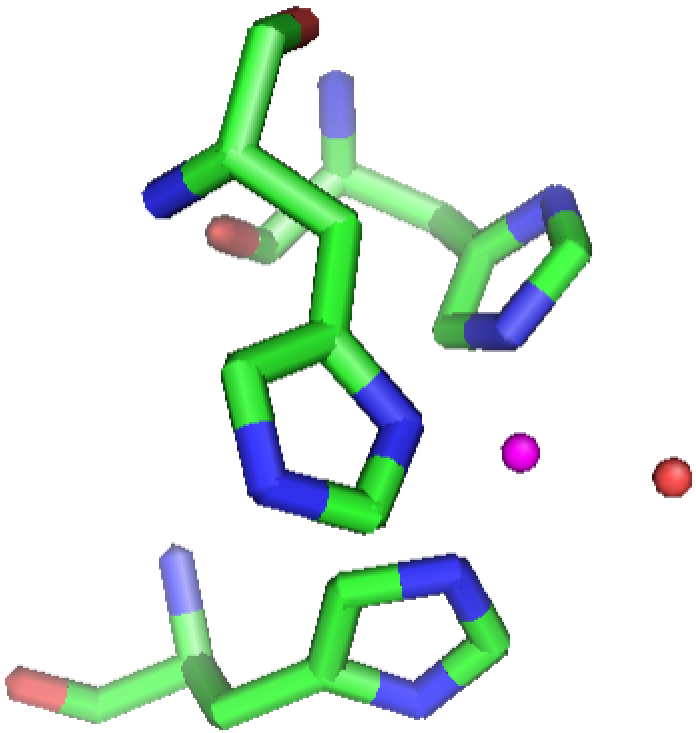
X-ray absorption (both metal and ligand edges) gives electronic structure information for open-shell complexes.



Carbonic Anhydrase

$$CO_2 + EZnOH^- \longleftrightarrow EZnHCO_3^- \overset{H_2O}{\longleftrightarrow} EZnH_2O + HCO_3^-$$

$$EZnH_2O + B \longleftrightarrow EZnOH^- + BH^+$$

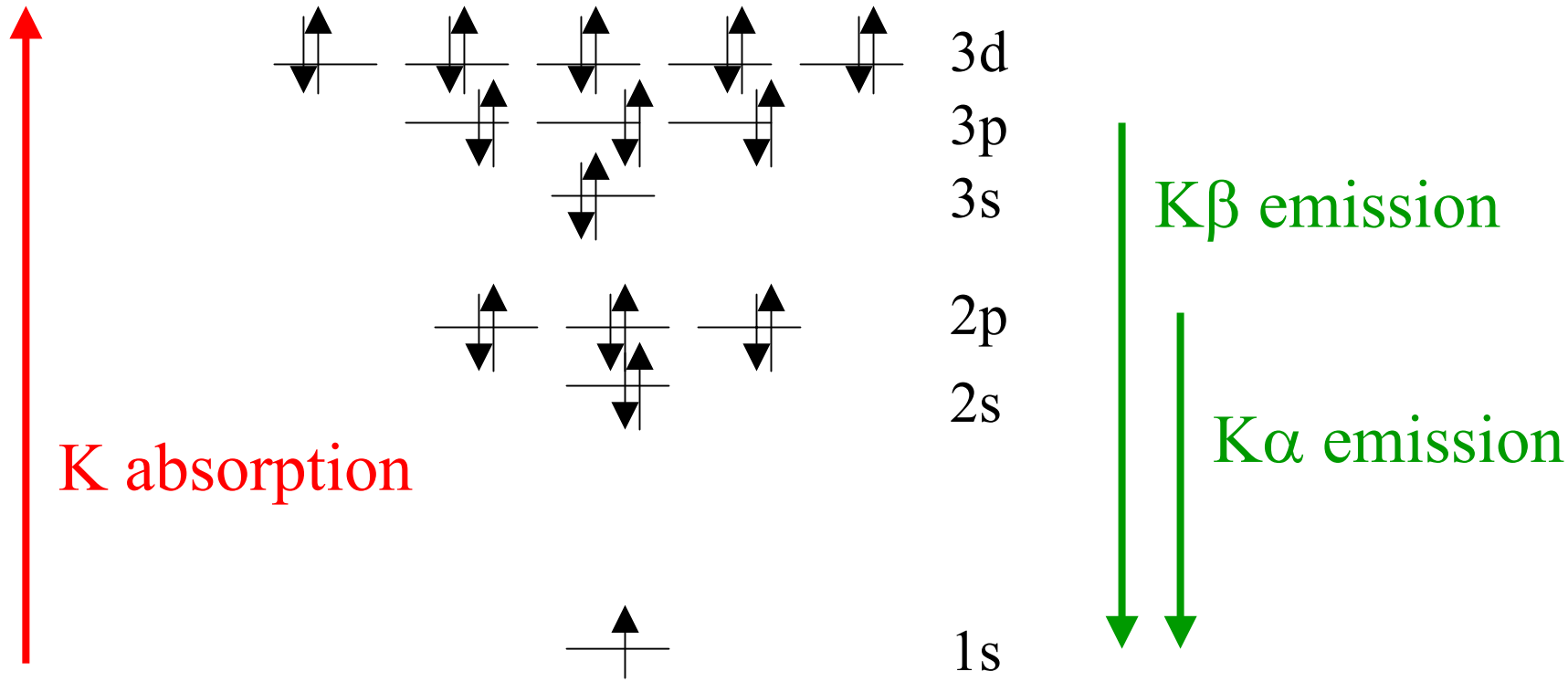


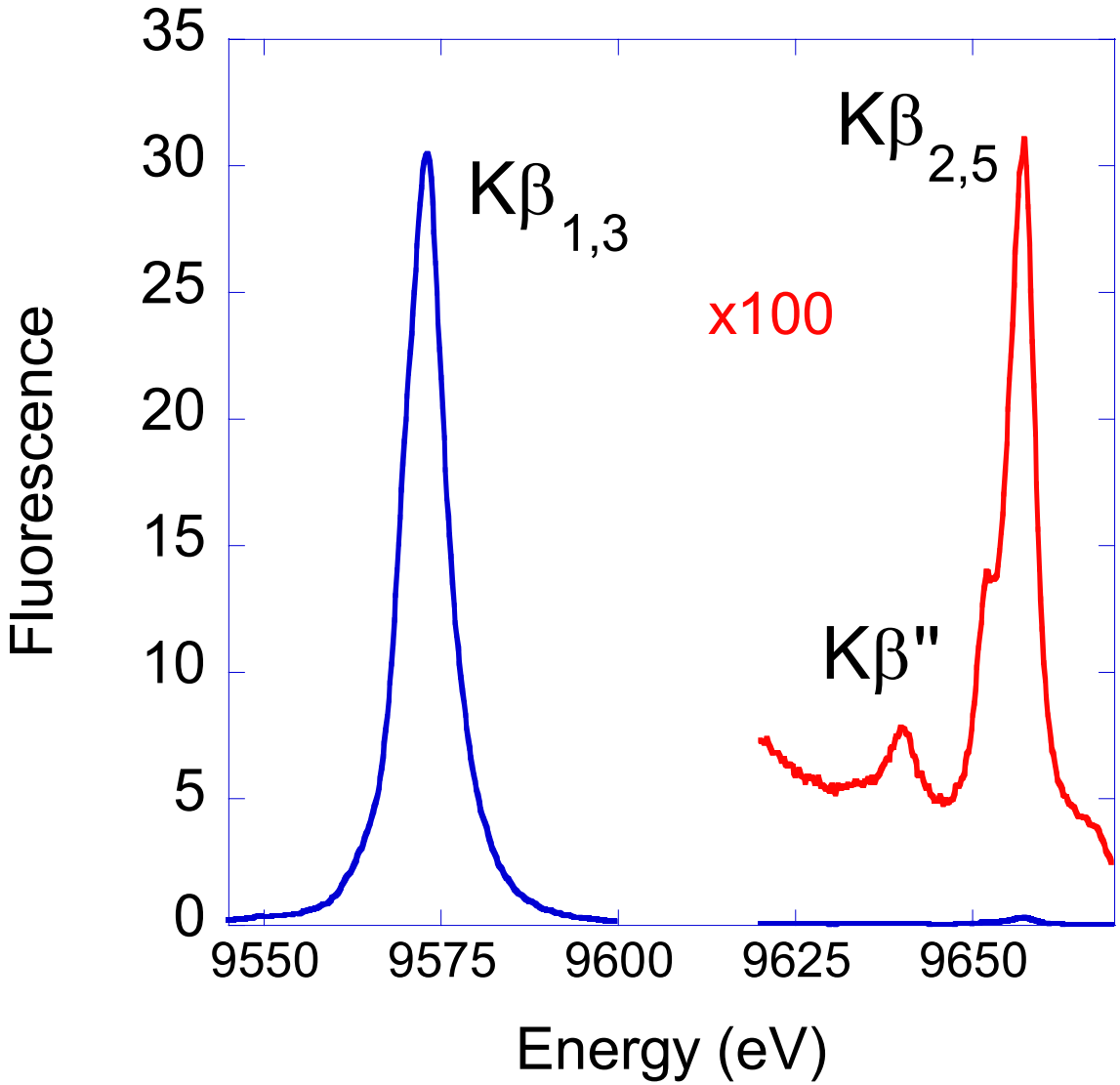
~~Zn-bound water~~ or

Zn-bound hydroxide?

Lipton et al., *J. Am. Chem Soc.* (2004), **126**, 4735-4739

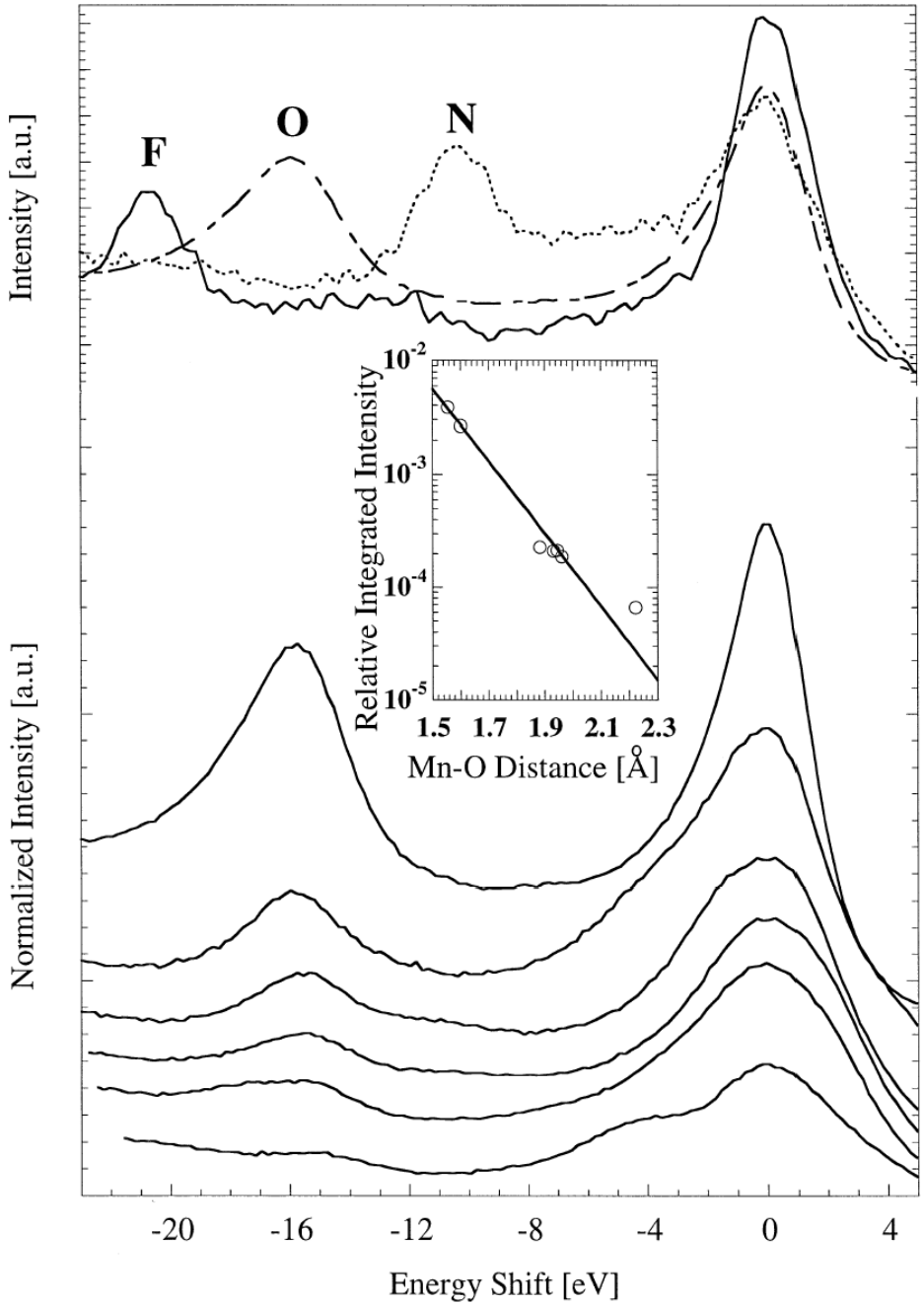
X-ray emission spectroscopy (XES) probes filled orbitals




35
30
25
20
15
10
5
0
Fluorescence
9550
9575
9600
9625
9650
Energy (eV)
Kβ1,3
Kβ2,5
x100
Kβ"

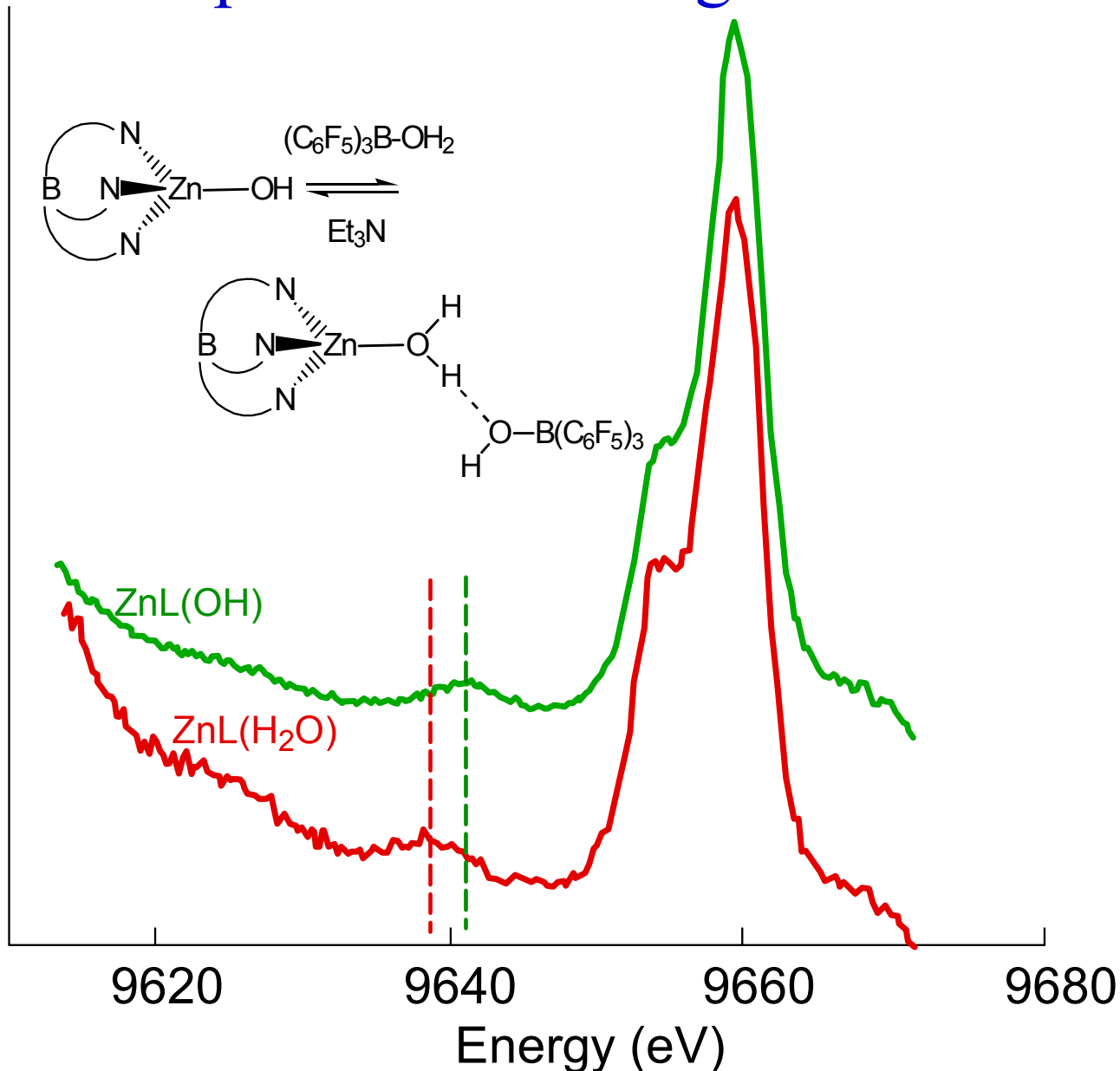
Ligand identification

"Cross-over" bands (Kβ") are due to ligand-metal charge transfer

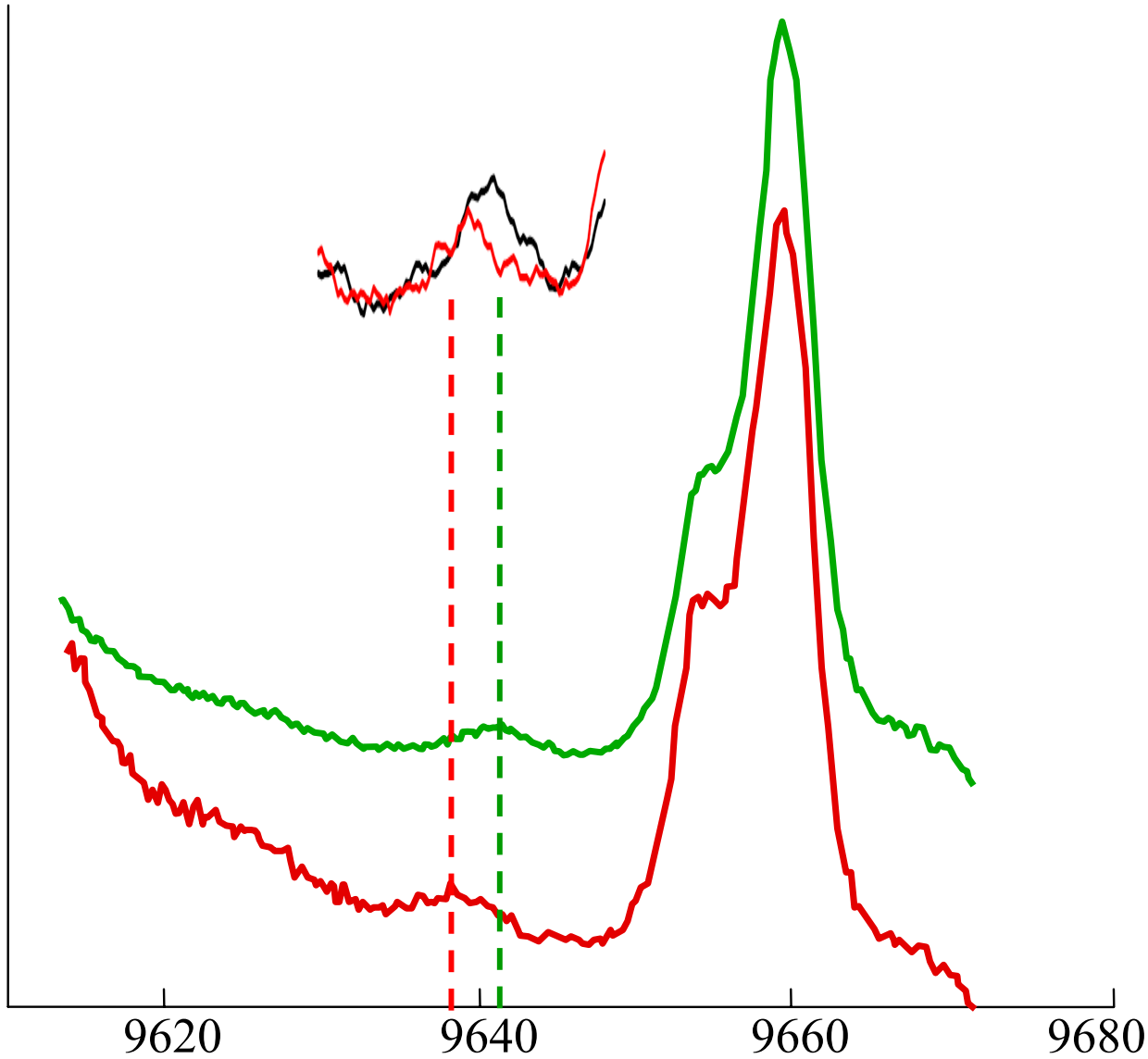


Bergmann et al., *Chem. Phys. Lett.* **1999** *302* 119

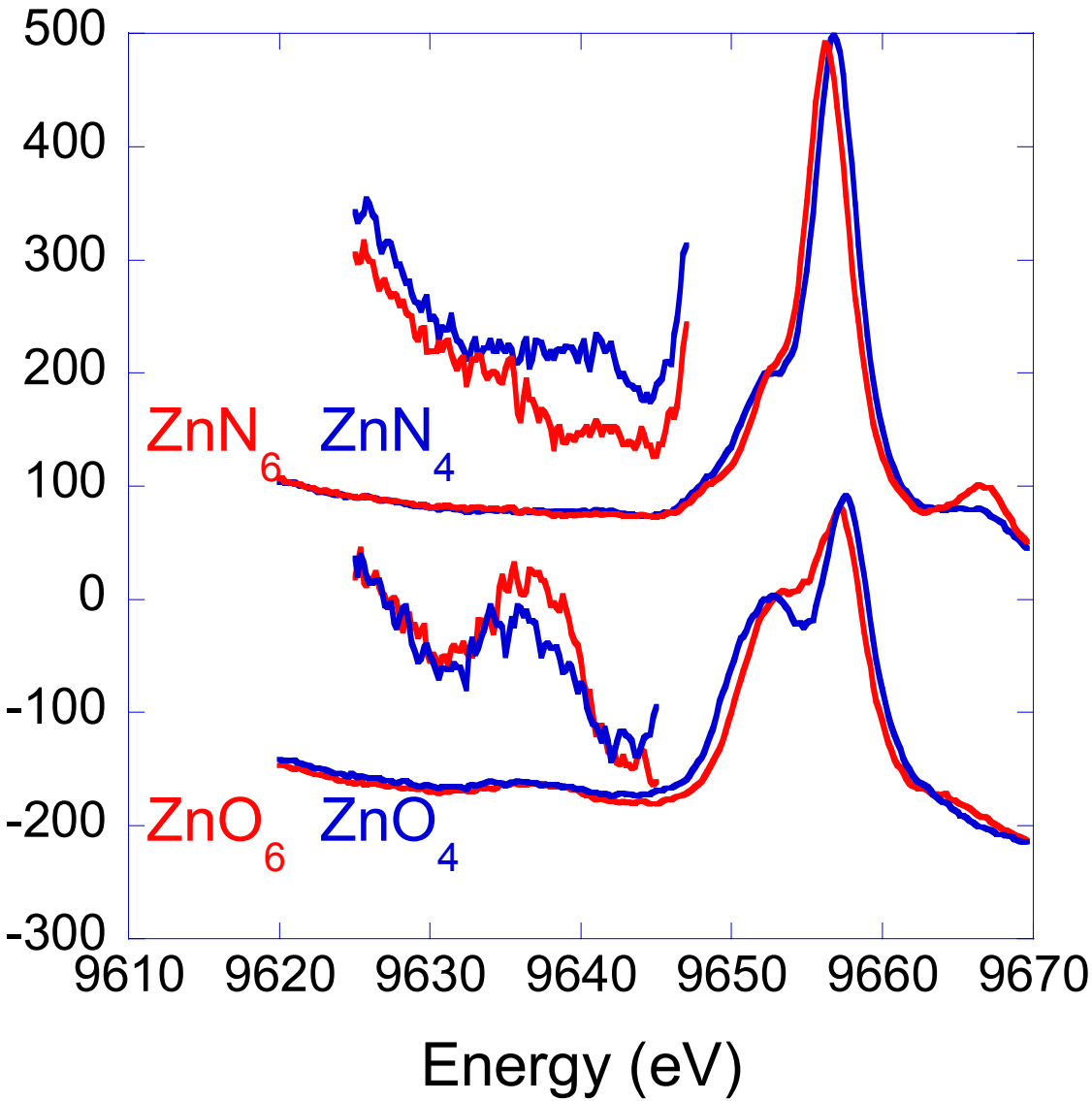
Cross-over band for Zn-O depends on protonation of ligand



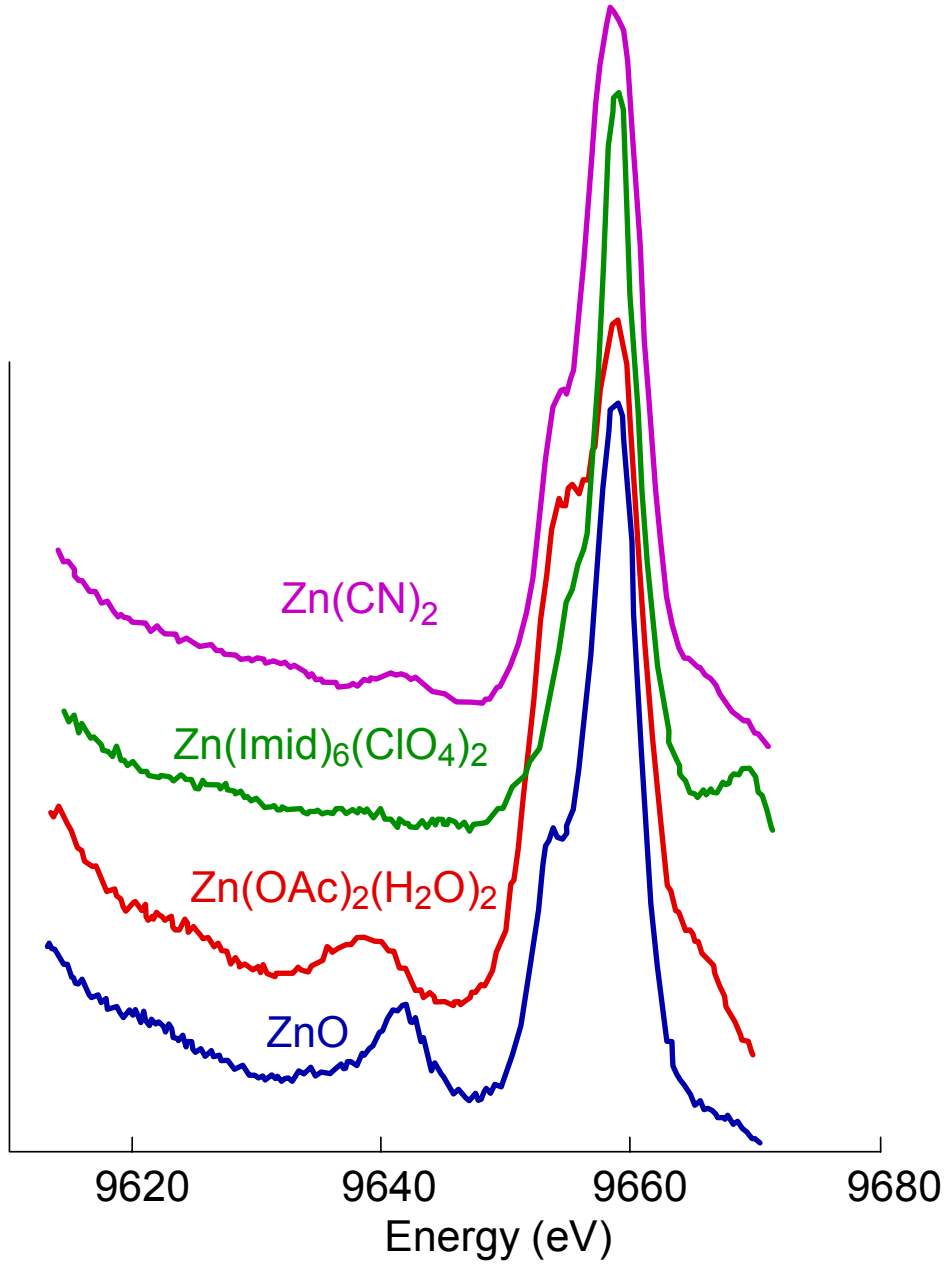
CA shows the same pH dependence as the models



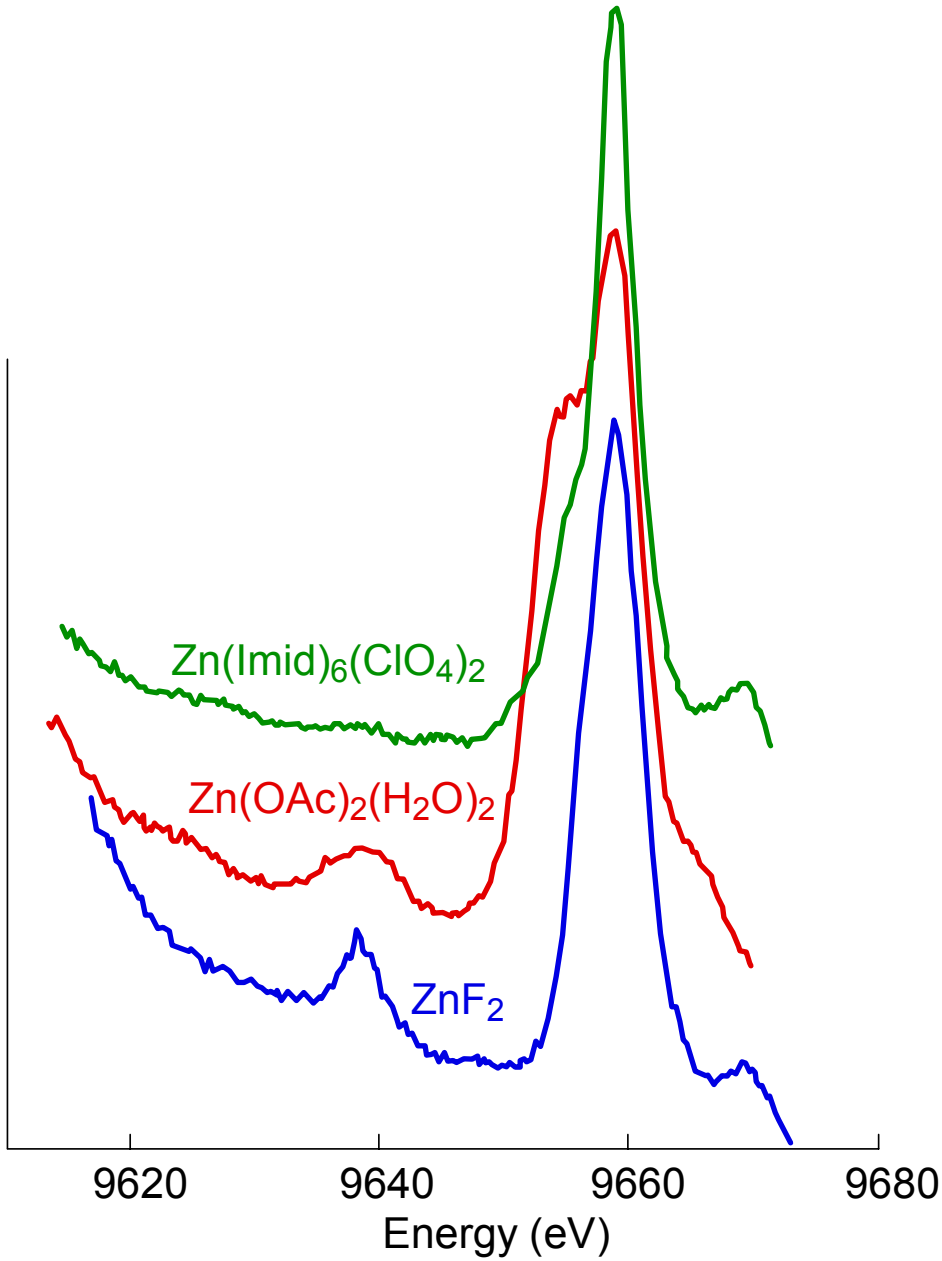
For a single ligand type (acetate or imidazole) cross-over peak is reproducible



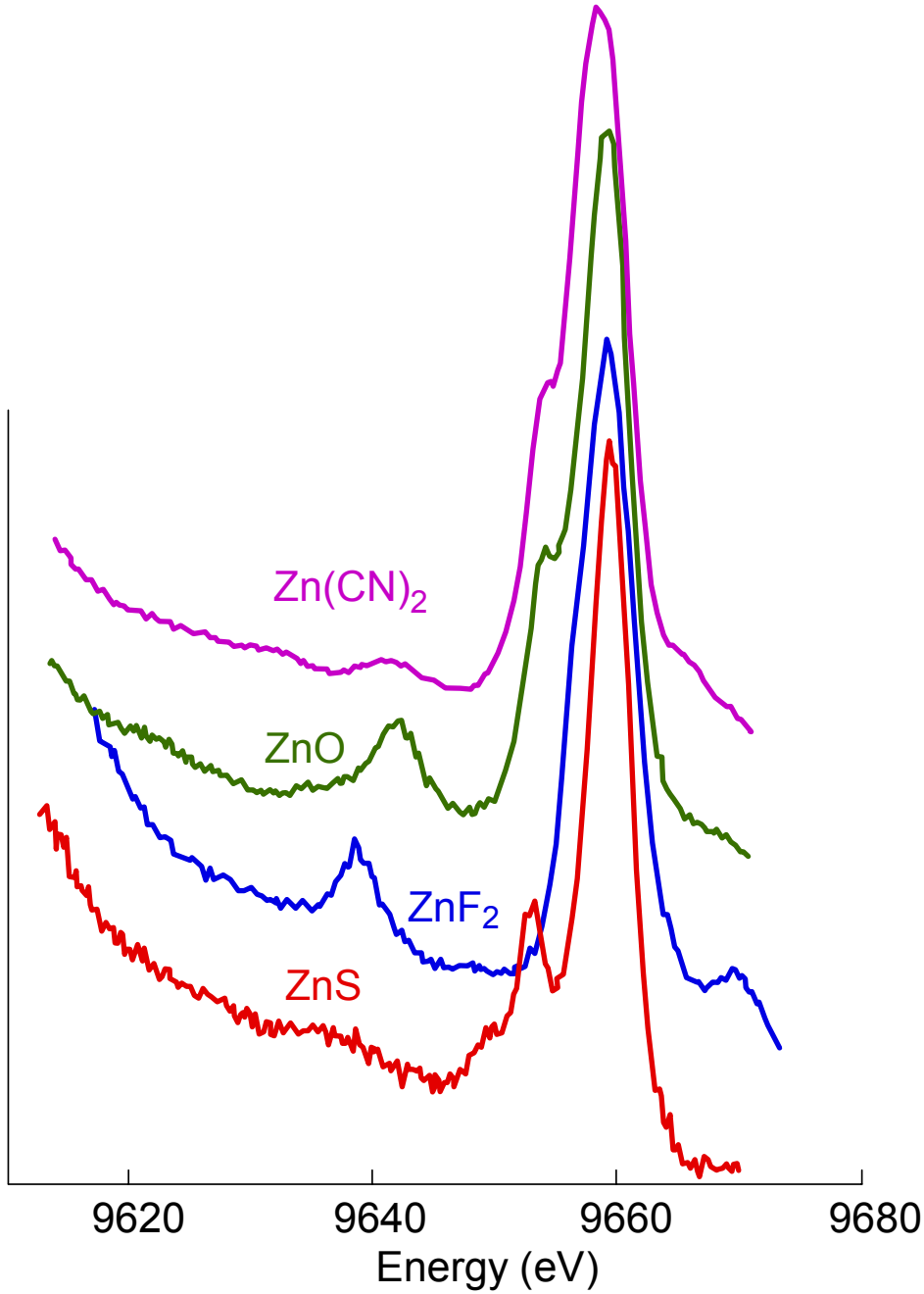
However, there is significant variation for different ligands with the same ligand atom



For octahedral Zn, cross-over energy is nearly independent of ligand identity

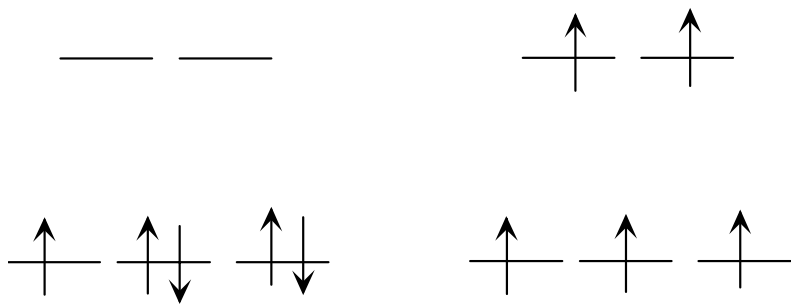


However, tetrahedral Zn does not follow the same pattern

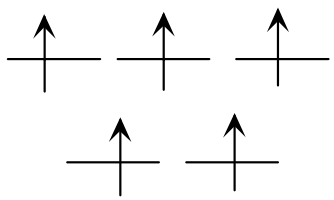


For open shell complexes, XANES can provide information about geometry

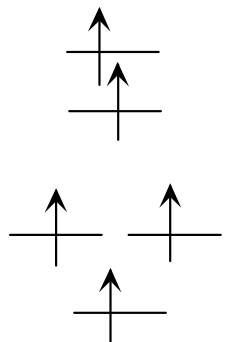
Octahedral



Tetrahedral

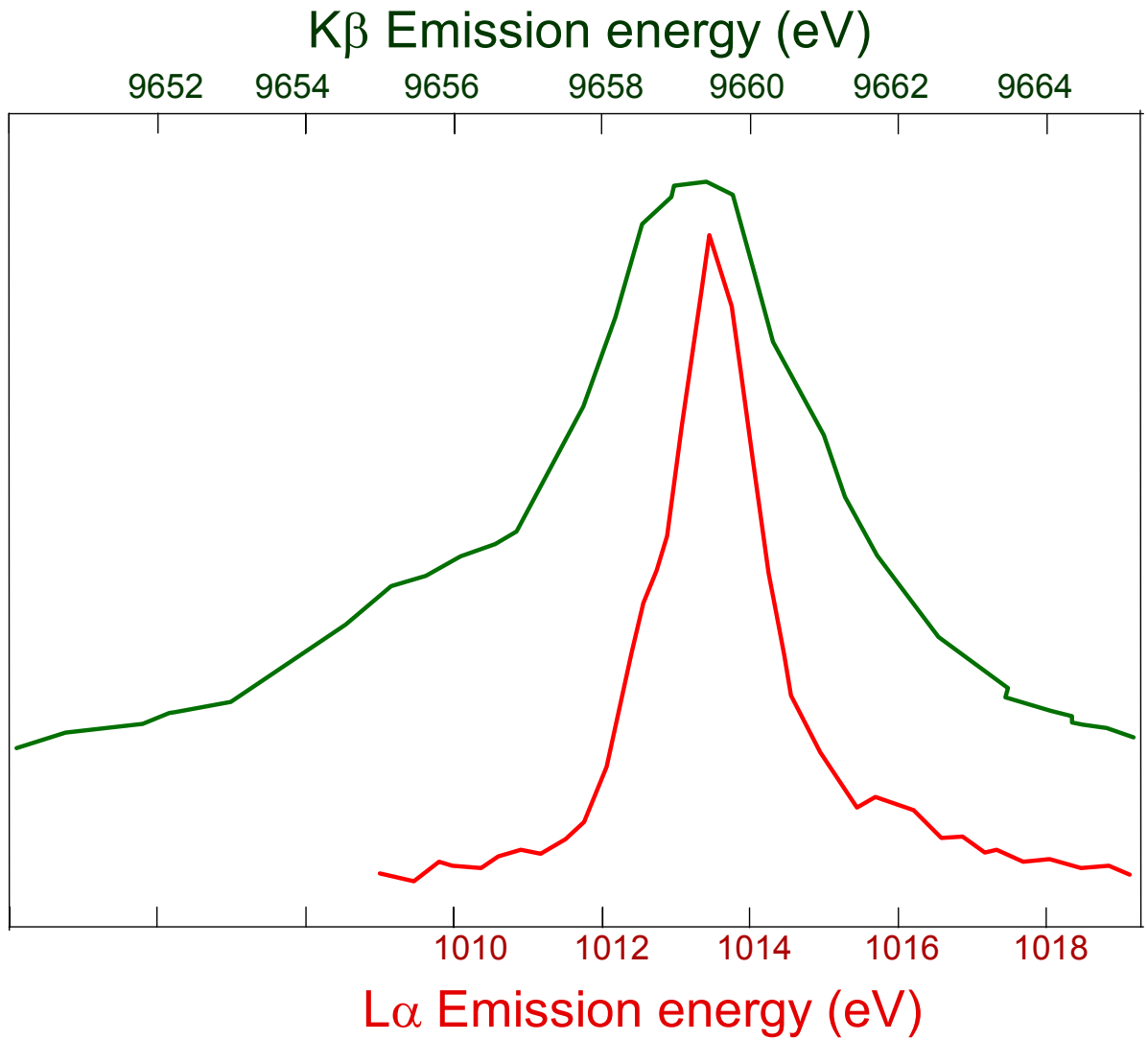


Square Planar



ZnO L edge emission

- Narrower
- Allowed 3d-2p



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